RESEARCH COMMUNICATION

2R of Thymidylate Synthase 5'-untranslated Enhanced Region Contributes to Gastric Cancer Risk: a Meta-analysis

Zhen Yang*, Hong-Xiang Liu, Xie-Fu Zhang

Abstract

Background: Studies investigating the association between 2R/3R polymorphisms in the thymidylate synthase 5'-untranslated enhanced region (TYMS 5'-UTR) and gastric cancer risk have generated conflicting results. Thus, a meta-analysis was performed to summarize the data on any association. Methods: Pubmed, Embase, and CNKI databases were searched for all available studies. The strength of association between TYMS 5'-UTR 2R/3R polymorphism and gastric cancer risk was estimated by odds ratios (ORs) with 95% confidence intervals (CIs). Results: Six individual case-control studies with a total of 1,472 cases and 1,895 controls were included into this meta-analysis. Analyses of total six relevant studies showed that there was no obvious association between the TYMS 5'-UTR 2R/3R polymorphism and gastric cancer risk. Subgroup analyses based on ethnicity showed 2R of TYMS 5'-UTR 2R/3R contributes to gastric cancer risk in the Asian population ($OR_{Homozygote\ model}$ = 1.71, 95% CI 1.19-2.46, P = 0.004; $OR_{Recessive\ genetic\ model}$ = 1.70, 95% CI 1.18-2.43, P = 0.004). However, the association in Caucasian populations was uncertain due to the limited studies. Conclusions: Our meta-analysis suggests that 2R of TYMS 5'-UTR 2R/3R contributes to gastric cancer risk in the Asian population, while this association in Caucasians populations needs further study.

Keywords: Gastric cancer - thymidylate synthase - polymorphism - meta-analysis - Asians - Caucasians

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Introduction

Gastric cancer (GC) was the sixth most common cancer worldwide (989, 600 new cancer cases) and the second most frequent cause of cancer death worldwide (738,000 cancer deaths) in 2008 (Jemal et al., 2011). Over 70% of new cases and deaths occur in developing countries, and the highest incidence rate is in Eastern Asia (Jemal et al., 2011). As a complex and multi-factorial process, the gastric carcinogenesis is still not fully understood. Epidemiological studies have revealed that Helicobacter pylori, smoking, diets and environmental risk factors play important roles in the development of GC (Hartgrink et al., 2009; Resende et al., 2010; Wroblewski et al., 2010). However, only a small proportion of individuals exposed to the known risk factors develop GC, while many cases develop GC among individuals without those risk factors, which suggest genetic factors also play an important role in GC etiology (Vogelstein and Kinzler, 2004; Hartgrink et al., 2009).

Thymidylate synthase (TYMS) is a critical enzyme in maintaining a balanced supply of deoxynucleotides required for DNA synthesis and repair, and is known to be involved in folate metabolism, which is one of the constituents in fruits and vegetables and may provide protection against GC (Hardy et al., 1987; Carreras and Santi, 1995). The TYMS gene is located on chromosome 18p11.32 and catalyzes the conversion of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP) using the 5, 10-methylenetetrahydrofolate as a methyl donor (Trinh et al., 2002). Thymidylate deficiency may result in chromosomal breakage and fragile site induction, which may cause individual susceptibility to GC (Lin et al., 2007). The TYMS gene contains a series of 28 bp tandem repeats in the 5'-untranslated enhanced region (TSER), and the double repeats (2R) or triple repeats (3R) are most common and known to be involved in modulation of TYMS mRNA expression and are thought to influence TYMS mRNA expression and stability (Horie et al., 1995). Over the last decade, several studies have investigated the association between the TSER polymorphism and risk of GC, but the results were conflicting (Graziano et al., 2004; Zhang et al., 2004; Tan et al., 2005; Wang et al., 2005; Zhang et al., 2005; YIM et al., 2010). Such inconsistency could be due to the small effect of the TYMS 5'-UTR 2R/3R polymorphism on gastric cancer risk and the relatively small sample-size in each of the published studies. Thus, to establish a comprehensive picture of the relationship between TYMS 5'-UTR 2R/3R polymorphism on gastric cancer risk, we performed a meta-analysis of the published studies to summarize previous data and clarify this inconsistency.

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Materials and Methods

Identification and eligibility of relevant studies

We searched the literature from PubMed, EMBASE and the CBM to identify relevant and available published articles. The keywords and subject terms (''thymidylate synthase '' or ''TYMS'' or ''TS'') and (''gastric cancer'' or "stomach cancer") and ("polymorphism'' or "mutation"). The last search date was July 30, 2011. The language of the papers was not restricted. All references cited in these studies and previously published review articles were retrieved for additional eligible studies not indexed by MEDLINE. The following criteria were used to select the eligible studies: (1) a case–control study on the association between TYMS 5'-UTR 2R/3R polymorphism and GC risk; (2) identification of GC was confirmed histologically or pathologically; (3) an available genotype or allele frequency for estimating an odds ratio (OR) with a 95% confidence interval (CI); (4) a genotype distribution among the control populations consistent with Hardy–Weinberg Equilibrium (HWE). When authors reported two or more publications on possibly the same patient populations, only the most recent or complete study was included in the review to avoid overlap between the cohorts. The major reasons for exclusion of studies were: (1) family studies; (2) containing overlapping data; (3) review papers.

Data extraction

Two reviewers independently evaluated the final articles included into this meta-analysis, and disagreements were resolved by reaching a consensus among all authors. Data retrieved from the reports included the following: first author's name, publication year, country of origin, source of controls, racial decent of the study population (categorized as Caucasian population and Asian population), genotyping method, eligible and genotyped cases and controls, the number for each TYMS 2R/3R genotype, and the allele frequency of TYMS 2R/3R.

Quality score assessment

The quality of the studies was also independently assessed by the same two reviewers according to the predefined scale for quality assessment. These scores were based on both traditional epidemiological considerations and cancer genetic issues. Any disagreement was resolved by discussion between the two reviewers. Total scores ranged from 0 (worst) to 15 (best). Reports scoring < 10 were classified as "low quality", and those ≥ 10 as "high quality".

Statistical methods

The strength of association between TYMS 5'-UTR 2R/3R polymorphism and gastric cancer risk was estimated by Odds ratios (ORs) with 95% confidence intervals (CIs). Four different comparison models of ORs were calculated: the allele model (2R vs. 3R), the Homozygote comparison model (2R/2R versus 3R3R), the Recessive genetic comparison model (2R/2R versus 2R/2R+3R3R), and the Dominant genetic comparison model (2R/2R + 2R/2R versus 3R3R). The χ^2-based Q statistic was used to investigate the degree of heterogeneity between the studies, and a P value < 0.05 was interpreted as significant heterogeneity among the studies (Cochran, 1954). Besides, the I^2 index expressing the percentage of the total variation across studies due to heterogeneity was also calculated further assess the between-study heterogeneity (Higgins and Thompson, 2002; Higgins et al., 2003). I^2 values of 25, 50, and 75% were used as evidence of low, moderate, and high heterogeneity, respectively. If heterogeneity existed, the random effects model (the DerSimonian and Laird method) (DerSimonian and Laird, 1986), which yields wider confidence intervals, was adopted to calculate the overall OR value. Otherwise, the fixed effects model (the Mantel-Haenszel method) was used (Mantel and Haenszel, 1959). In order to assess the stability of the results, sensitivity analyses were performed by reanalyzing the significance of ORs after omitting each study in turn (Tobias, 1999). Funnel plots and Egger's linear regression test were used to assess evidence for potential publication bias (Egger et al., 1997; Stuck et al., 1998). The analysis was conducted using version 10.0 of STATA (Biostat, NJ, USA).

Results

Characteristics of studies

The combined search yielded 121 references. After discarding overlapping references and those which clearly did not meet the criteria, six studies were included into this meta-analysis (Graziano et al., 2004; Zhang et al., 2004; Tan et al., 2005; Wang et al., 2005; Zhang et al., 2005; YIM et al., 2010). As shown in Table 1, six case-control studies including a total of 1, 472 cases and 1, 895 controls finally

Table 1. Characteristics of Studies Included in This Meta-analysis

Study	Ethnicity	Country	Case group	Control group	P_{HWE}*	Quality score
Graziano F, 2004	Caucasians	Italy	132 patients with histologically confirmed gastric cancer	139 healthy controls recruited from population	0.44	14
Zhang J, 2004	Asians	China	232 patients with histologically confirmed gastric cardiac adenocarcinoma	347 healthy controls recruited from population	0.93	14
Zhang Z, 2005	Asians	China	337 patients with histologically confirmed gastric cancer	326 cancer-free control subjects	0.65	12
Tan W, 2005	Asians	China	324 patients with histologically confirmed gastric cancer	492 healthy controls recruited from normal population	0.38	13
Wang LD, 2006	Asians	China	129 patients with histologically confirmed gastric cancer	315 cancer-free controls	0.05	10
Yim DJ, 2010	Asians	Korea	318 patients with histologically confirmed gastric cancer	280 healthy controls recruited from normal population	0.36	12

Table 2. Summary of Pooled Odds Ratios (OR) with Confidence Interval (CI) in the Meta-analysis

Comparison Model	Studies (No. of cases / controls)	Odds Ratio OR[95%CI]	P_{OR}	M*	Heterogeneity I^2 (%)	P_H †
All studies						
2R vs. 3R	6(1472/1895)	1.08(0.96- 1.22)	0.203	F	15.8	0.312
Homozygote comparison model	6(1472/1895)	1.42(0.85- 2.36)	0.176	R	56.7	0.042
Recessive genetic comparison model	6(1472/1895)	1.42(0.84- 2.38)	0.192	R	62.3	0.023
Dominant genetic comparison model	6(1472/1895)	1.06(0.92- 1.23)	0.421	F	0	0.746
Asians						
2R vs. 3R	5(1340/1756)	1.14(1.00-1.29)	0.129	F	0	0.827
Homozygote comparison model	5(1340/1756)	1.71(1.19-2.46)	0.004	F	0	0.452
Recessive genetic comparison model	5(1340/1756)	1.70(1.18-2.43)	0.004	F	1.8	0.396
Dominant genetic comparison model	5(1340/1756)	1.09(0.93- 1.26)	0.288	F	0	0.814
Caucasian						
2R vs. 3R	1 (132/139)	0.77(0.55-1.08)	0.052	F	NA	NA
Homozygote comparison model	1 (132/139)	0.52(0.25- 1.09)	0.084	F	NA	NA
Recessive genetic comparison model	1 (132/139)	0.55(0.29- 1.04)	0.066	F	NA	NA
Dominant genetic comparison model	1 (132/139)	0.80(0.47-1.37)	0.421	F	NA	NA

*M, model of meta-analysis; R, random-effects model; F, Fixed-effects model; †P_H, the P value of heterogeneity test

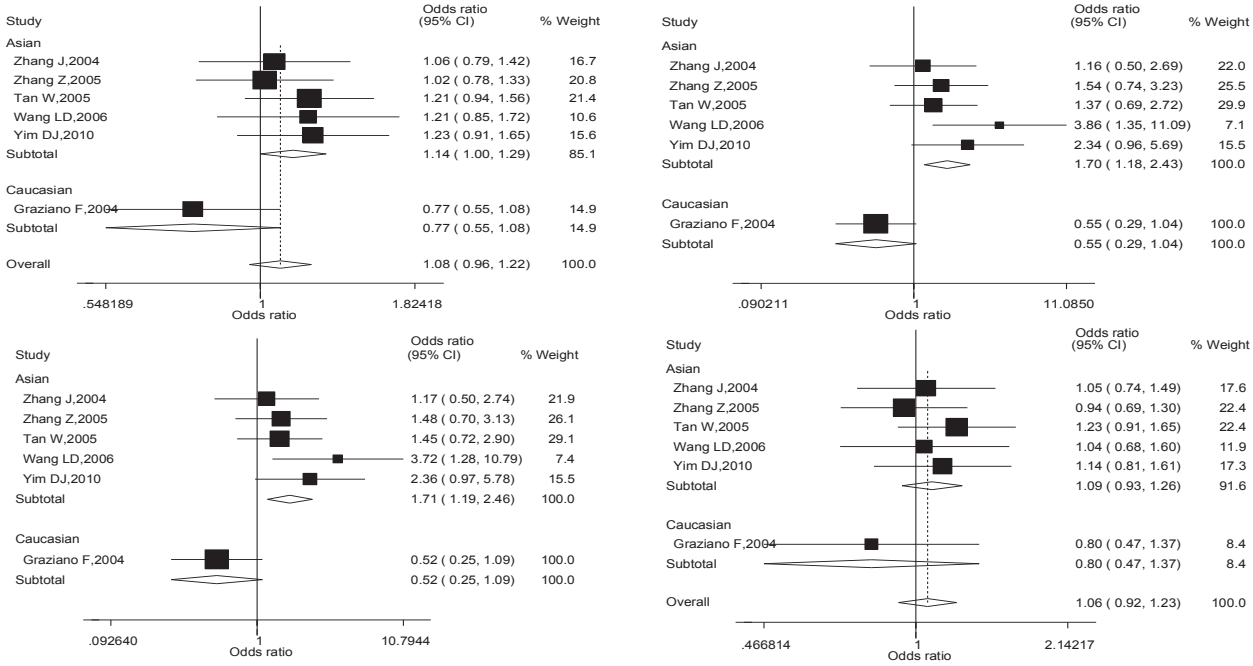

Figure 1. Forest Plot of Pooled OR with 95% CI for TSER 2R/3R Polymorphism and GC Risk (A, 2R vs.3R, Fixed effects model; B, Homozygote comparison model, Random effects model; C, Recessive genetic comparison model, Random effects model; D, Dominant genetic model, Random effects model) (The squares and horizontal lines corresponded to the study-specific OR and 95% CI. The area of the squares reflected the study-specific weight (inverse of the variance). The diamond represented the pooled OR and 95% CI)

met our criteria for inclusion. The detailed characteristics of these studies are summarized in Table 1. There were five case-control studies from Asian population (a total of 1, 340 cases and 1, 756 controls), while only one study was from Caucasian population (132 cases and 139 controls). All these 6 studies were high quality (Table 1).

Meta-analysis results

The results of this meta-analysis were shown in Table 2. The between-study heterogeneity was significant in the analyses of both homozygote comparison model and recessive genetic comparison model, and the random-effects model was preformed; while the between-study heterogeneity was not obvious in the other comparison models, and the fixed-effects model was preformed. Analyses of total six relevant studies showed that there was no obvious association between TYMS 5'-UTR 2R/3R polymorphism and gastric cancer risk (Table 2). In addition, sensitivity analysis indicated that single study could influence the pooled OR qualitatively, suggesting that the result was not stable.

Subgroup analyses were performed based on ethnicity including Asian population and Caucasian population. In the subgroup analyses of Asian population, there was no between-study heterogeneity in all comparison models, and the fixed-effects model was preformed to pool the results. Subgroup analyses in Asian population showed 2R of Thymidylate synthase 5'-untranslated enhanced region contributes to gastric cancer risk ($OR_{\text{Homozygote model}}$ =1.71, 95%CI=1.19-2.46, P=0.004; $OR_{\text{Recessive genetic model}}$ =1.70, 95%CI=1.18-2.43, P=0.004) (Figure 2). In addition, sensitivity analysis indicated that no single study could

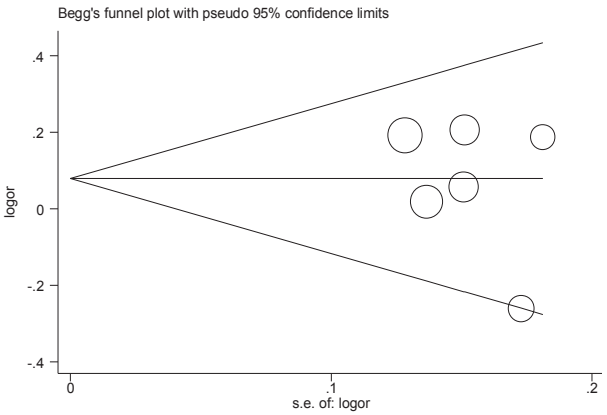

Figure 2. Funnel Plot for Publication Bias Test in the Meta-analysis Investigating the Association Between TSER 2R/3R Polymorphism and GC Risk

influence the pooled OR qualitatively, suggesting that the result was stable in the subgroup analyses of Asian population.

As to the subgroup analyses of Caucasian population, there was only one study and the outcomes from this study showed that there was no obvious association between TYMS 5'-UTR 2R/3R polymorphism and gastric cancer risk in the Caucasian population.

Publication bias

Funnel plot and Egger's test were used to assess publication bias. The shape of the funnel plots was symmetrical, and the Egger test provided evidence that there was no publication bias among the studies included ($T_{\text{Egger test}}$=-0.77, 95%CI=-13.8~7.8, P = 0.482). Thus, the publication bias was not obvious in this meta-analysis.

Discussion

Recent studies showed that functional polymorphisms in the TYMS gene may result in alterations in TYMS enzyme efficiency and/or expression level and may contribute to different cancers' risk via effects on nucleotide synthesis (Wang et al., 2010). Considering the potential influence of altering TYMS activation on folate metabolism, many epidemiological studies have explored the association between the TSER 2R/3R polymorphism and GC risk, but the results were conflicting (Graziano et al., 2004; Zhang et al., 2004; Tan et al., 2005; Wang et al., 2005; Zhang et al., 2005; YIM et al., 2010). Such inconsistency could be due to the small effect of the TSER 2R/3R polymorphism on GC risk or the relatively small sample-size in each of the published studies. Therefore, we performed a meta-analysis of 6 published case-control studies covering 1472 cases and 1895 controls to obtain a more precise estimation of the relationship between the TSER 2R/3R polymorphism and GC risk for the first time up to now. The results of meta-analyses showed that the 2R of TYMS 5'-UTR 2R/3R contributes to gastric cancer risk in the Asian population ($OR_{\text{Homozygote model}}$ =1.71, 95%CI=1.19-2.46, P = 0.004; $OR_{\text{Recessive genetic model}}$ =1.70, 95%CI 1.18-2.43, P = 0.004). However, as only one study was published in Caucasian population, the association in Caucasian population was still uncertain due to the study sample.

The 2R or 3R genetic variants are the most common genetic mutations of TSER gene and are known to be involved in the modulation of TYMS mRNA expression (Lin et al., 2007; Wang et al., 2010). The two alleles of TSER gene differ not only biologically but also functionally in their ability to alter TYMS activation on folate metabolism (Marsh et al., 2001; Ho et al., 2011). Thus, there is obvious biological evidence for the different effects on cancer development between the two different variants. In addition, our pooled analysis adds strong epidemiological evidence for the association between the TSER 2R/3R polymorphism and GC risk. Thus, biological evidence and epidemiological evidence both confirm the association between the TSER 2R/3R polymorphism and GC risk.

However, some possible limitations in our meta-analysis should be acknowledged. Firstly, the eligibility criteria for inclusion of controls were different from each other (Graziano et al., 2004; Zhang et al., 2004; Tan et al., 2005; Wang et al., 2005; Zhang et al., 2005; YIM et al., 2010). The controls in some studies were selected from non-cancer patients, while the controls in other several studies were just selected from asymptomatic individuals (Graziano et al., 2004; Zhang et al., 2004; Tan et al., 2005; Wang et al., 2005; Zhang et al., 2005; YIM et al., 2010). Additionally, misclassification bias was possible. For example, most studies could not exclude latent CRC cases in the controls (Graziano et al., 2004; Zhang et al., 2004; Tan et al., 2005; Wang et al., 2005; Zhang et al., 2005; YIM et al., 2010). Finally, gene-gene and gene-environmental interactions were not fully addressed in this meta-analysis for the lack of sufficient data. As we know, aside from genetic factor, smoking is a major risk factor for CRC; however we didn't perform subgroup analyses in smokers or nonsmokers owing to the limited reported information on such associations in the included studies.

Despite of those limitations, this meta-analysis suggests 2R of TYMS 5'-UTR 2R/3R contributes to gastric cancer risk in the Asian population, while this association in Caucasian population needs further study.

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The authors declare that they have no competing interests.

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